DOE Hydrogen Program

Fermentative and Electrohydrogenic Approaches to Hydrogen Production

2008 DOE Hydrogen Program Review

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June 11, 2008

Project ID PDP27

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Overview

DOE Hydrogen Program

Timeline

- Project start date: FY05
- Not funded in FY06
- Project end date: continuing
- Percent complete: N/A

Barriers

- Production Barriers addressed
  - Barrier AR: H_2 molar yield
  - Barrier AT: glucose feedstock cost

Budget

- Total project funding
  - $1,180K
- Funding received in FY07: $500K (including $130K subcontract to Penn State)
- Funding for FY08: $680

Partners

- Prof. Bruce Logan, Penn State Univ. (subcontract)

Objectives

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- The long-term objective is to develop direct fermentation technologies to convert renewable lignocellulosic biomass resources to H_2

- The near-term objectives in FY08 are to
  - Optimize bioreactor performance for the cellulose-degrading bacterium *Clostridium thermocellum*
  - Identify key metabolic pathways to guide genetic engineering to improve H_2 molar yield
  - Integrate microbial electrolysis cell (MEC) (formerly BEAMR: bio-electrochemically assisted microbial reactor) process to improve H_2 molar yield

Milestones

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Month/year	Milestones
September - 07	Optimize growth conditions for *Clostridium thermocellum* 27405 (FY2007 project start date is April 2007) (NREL)
April - 08	Test H_2 production in a microbial electrolysis cells (MEC) using synthetic solution having the same composition as that produced from the NREL lignocellulose fermentation system (PSU)
June – 08	Test effects of metabolic pathway inhibitors on H_2 production (NREL)
August - 08	Determine H_2 molar yield and mass balance of fermentation using pretreated biomass as the feedstock (NREL)

Approaches

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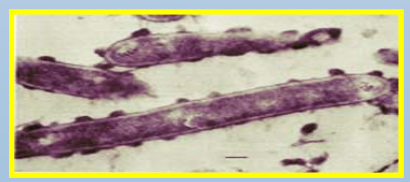
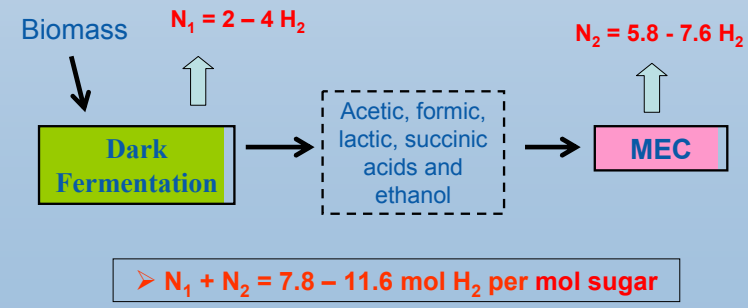
- **Task 1:Bioreactor Performance**
  - Optimize cellulose-degrading bacterium *Clostridium thermocellum* 27405 to lower feedstock cost by converting cellulose to H_2 directly
- **Task 2: Metabolic Engineering**
  - Use genetic tools to improve the metabolic pathway of *C. thermocellum* (genome sequenced) to increase H_2 yield
- **Task 3: Microbial Electrolysis Cell** (Penn State).
  - Develop microbial electrolysis cells to produce H_2, using waste generated from the NREL fermentation system



Clostridium thermocellum

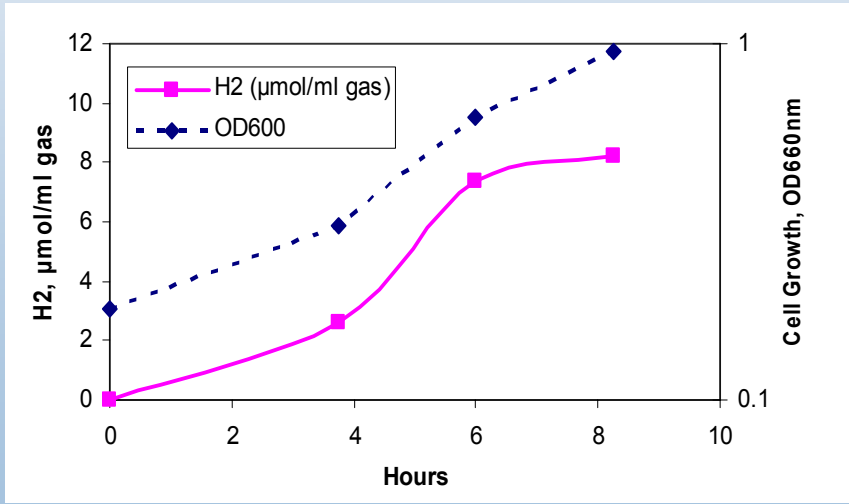


➢ $N_1 + N_2 = 7.8 – 11.6$ mol H_2 per mol sugar

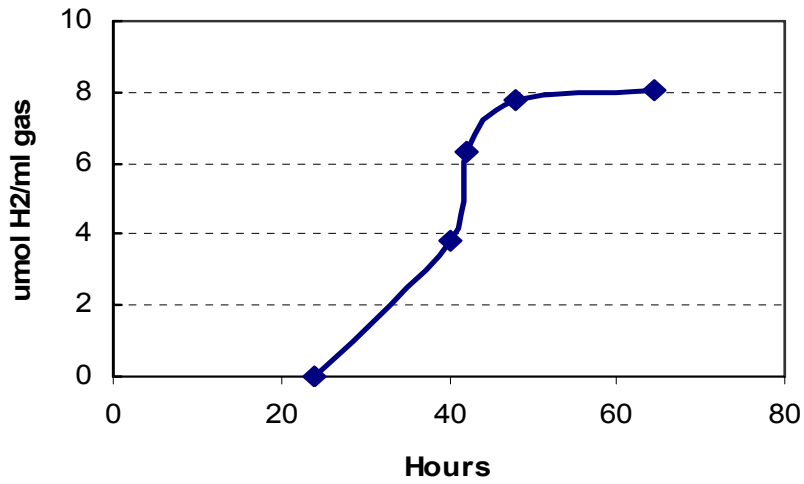
Technical Accomplishments:
Optimized Growth and H_2 Production

- **Task 1:** Growth of *C. thermocellum* was optimized and it displayed a cell-doubling time of 2 hrs at 55 °C, while converting various cellulosic substrates to H_2.

(A) cellobiose



(B) H_2 from Avicel Cellulose



Technical Accomplishments:
Optimized Growth and H_2 Production

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- **Task 1:** *Clostridium thermocellum* converting various cellulosic substrates to H_2.

Substrate*	μmol H_2/ml culture/day
Corn stover	**23.3**
Avicel cellulose	15.7
Cellobiose	11.4
Filter paper	4.2

*Added at 0.5% (w/v) except biomass at 1.4%

➢ Exceeding Milestone (09/07) in optimizing cell growth and cellulose utilization

Technical Accomplishments:
H_2 from Corn Stover in Bioreactor

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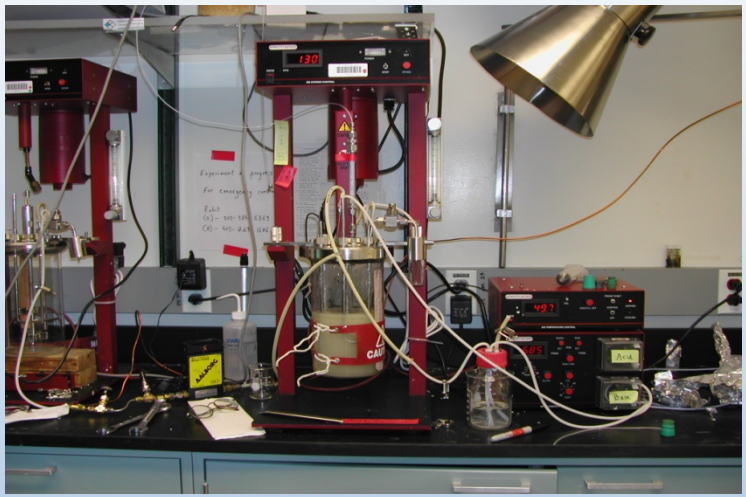
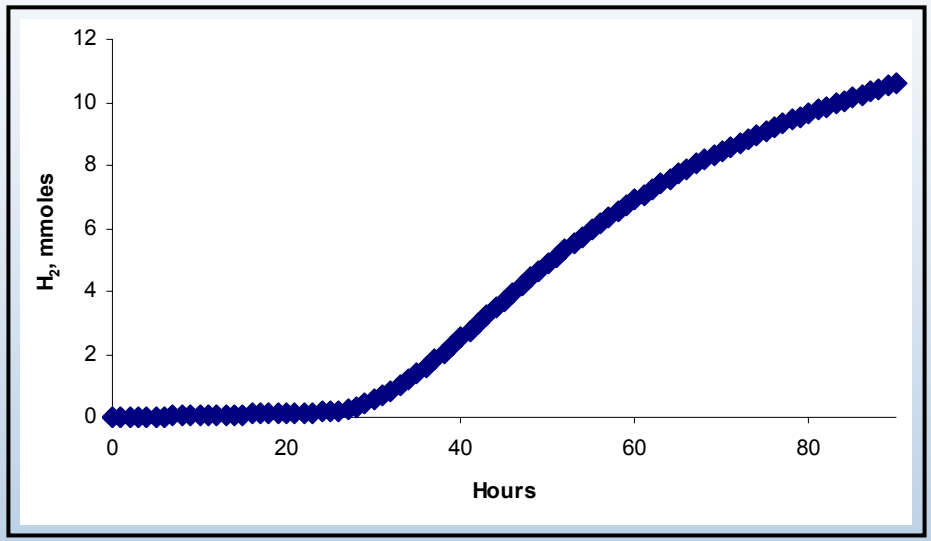
Task 1: Bioreactor performance using corn stover





- pH, temperature (50 °C), and pressure controls
- Continuous on-line measurements of H_2 and CO_2

Toward meeting Milestone (8/08)

- Corn stover lignocellulose prepared by acid hydrolysis in 1.1% H_2SO_4
- 0.14% (w/v) corn stover was completely consumed in the end of fermentation
- Metabolite profiles and H_2 molar yield determinations underway

Technical Accomplishments:
Increased H_2 Yield

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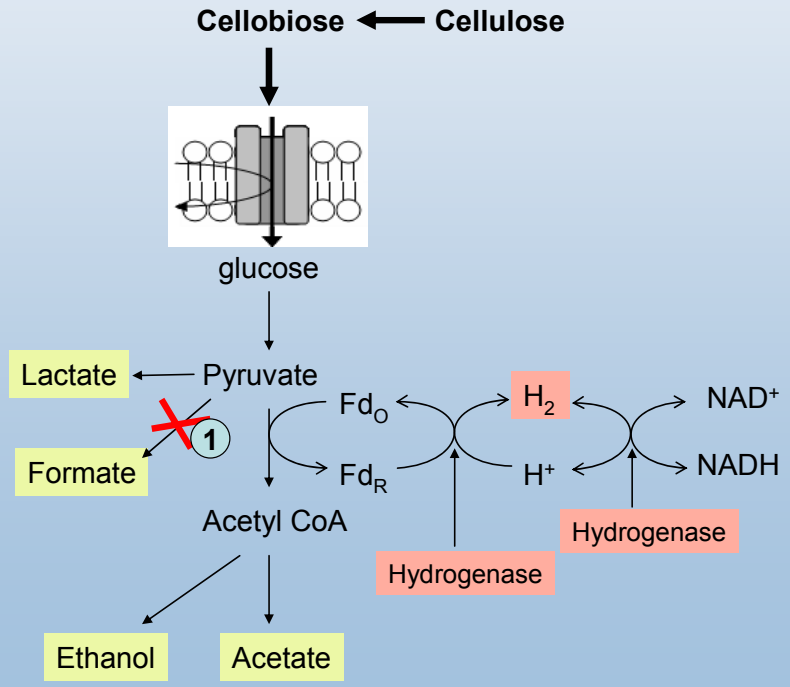
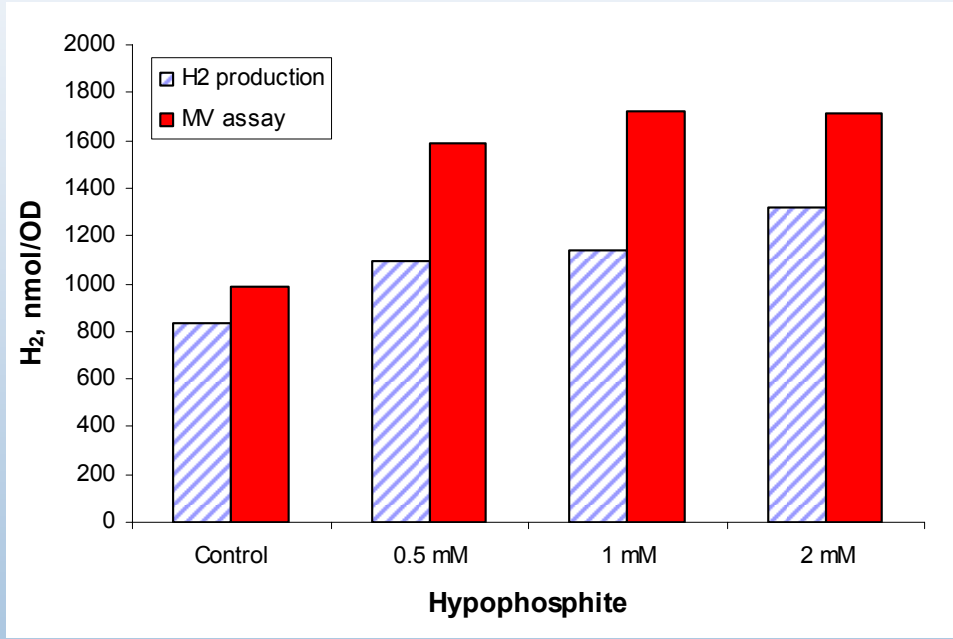
- **Task 2:** we studied effects of pathway inhibitors on H_2 production. The outcome will guide the most effective genetic engineering effort.
- Blocking the pyruvate-to-formate competing pathway by *hypophosphite* increased H_2 production.





- **Increased H_2 yield by 31% to 58%**
- Increased hydrogenase activity by 61-74%

Technical Accomplishments:
Increased H_2 Yield

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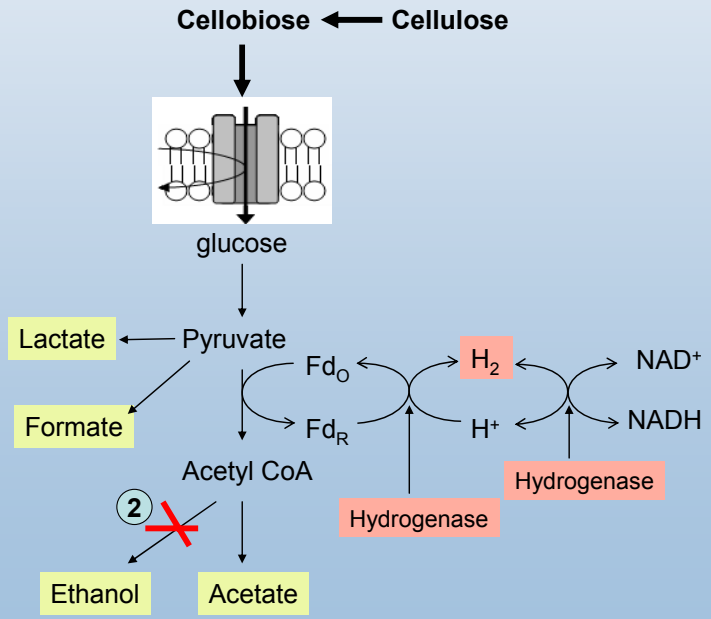
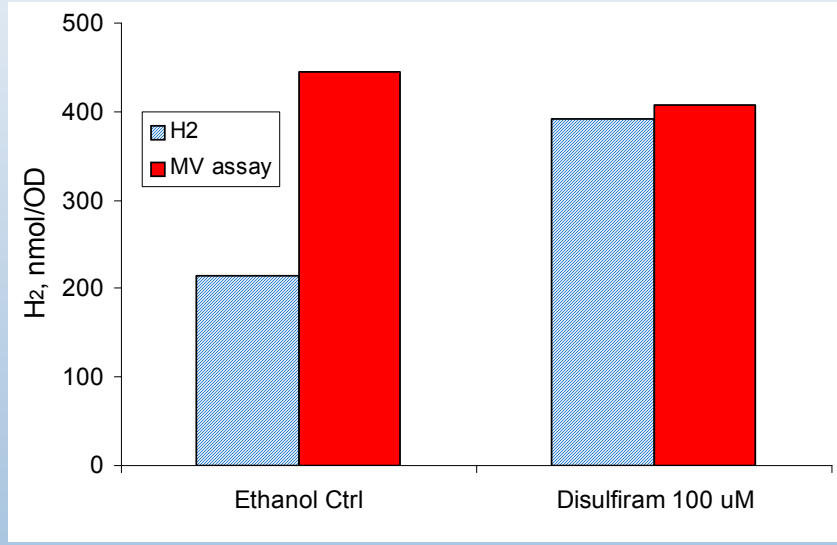
- **Cont'd Task 2:** Blocking the ethanol competing pathway by *disulfiram* increased overall H_2 production
- We demonstrated that blocking competing pathways is effective in increasing H_2 yield – Milestone (6/08)

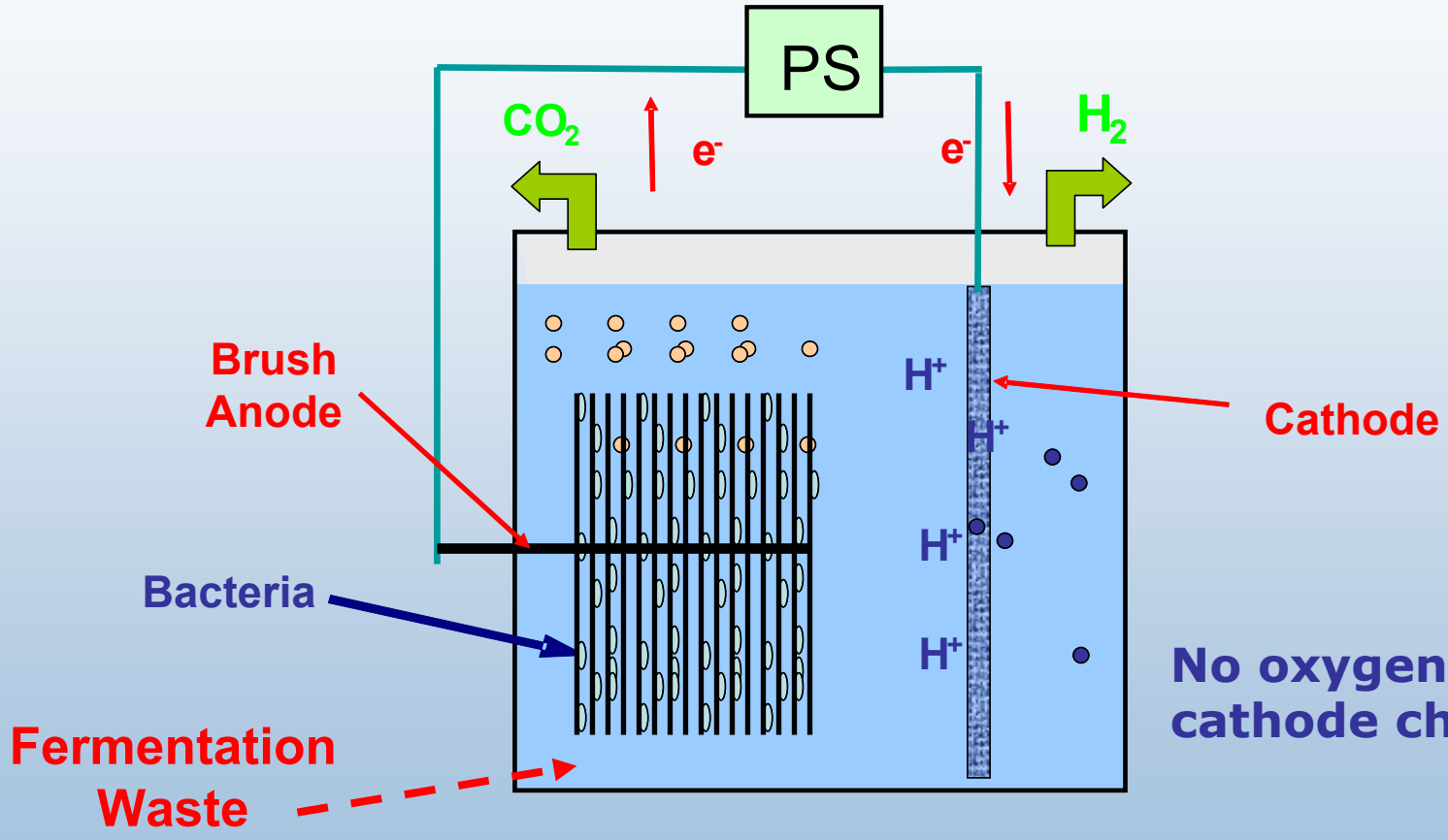




- **Increased H_2 yield by 81%**
- No change in hydrogenase activity

Task 3 Approach:

Microbial Electrolysis Cell (MEC)



No oxygen in cathode chamber

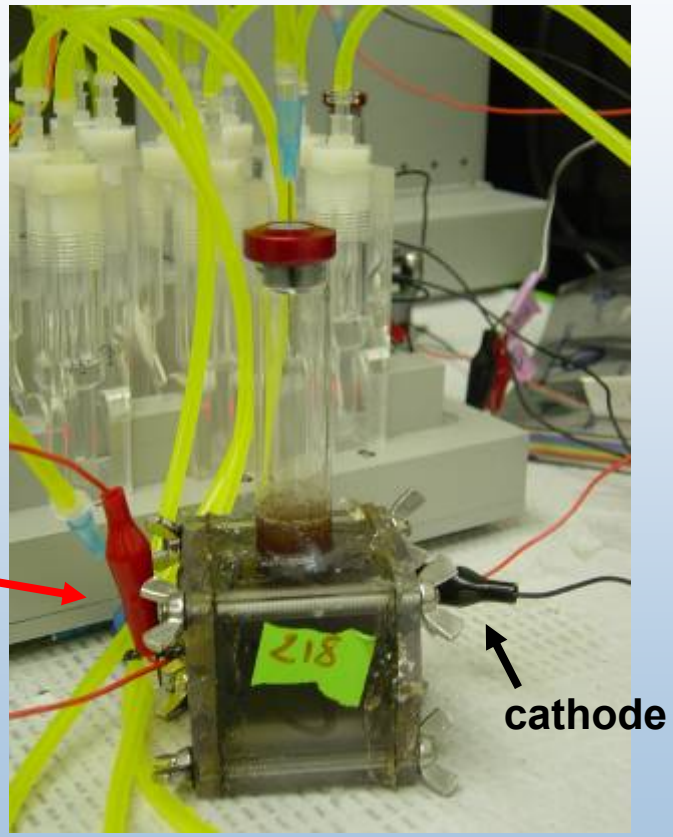
0.25 V needed (vs 1.8 V for water electrolysis)

Bruce Logan, Penn State University

Ref: Liu, Grot and Logan, *Environ. Sci. Technol.* (2005)

Task 3 Approach:

Reactor and Solutions



MEC used in tests (also called BEAMR)

- Reactor
  - Single chamber
  - Brush anode, carbon cathode with Pt
- Synthetic solution containing fermentation end products:
  - Substrates:
    - 26 mM acetic acid
    - 5.6 mM succinic acid
    - 1.8 mM lactic acid
    - 0.6 mM formic acid
    - 14 mM ethanol
  - 50 mM PBS + vitamins + minerals

Technical Accomplishments:
H_2 from Synthetic Fermentation End Products

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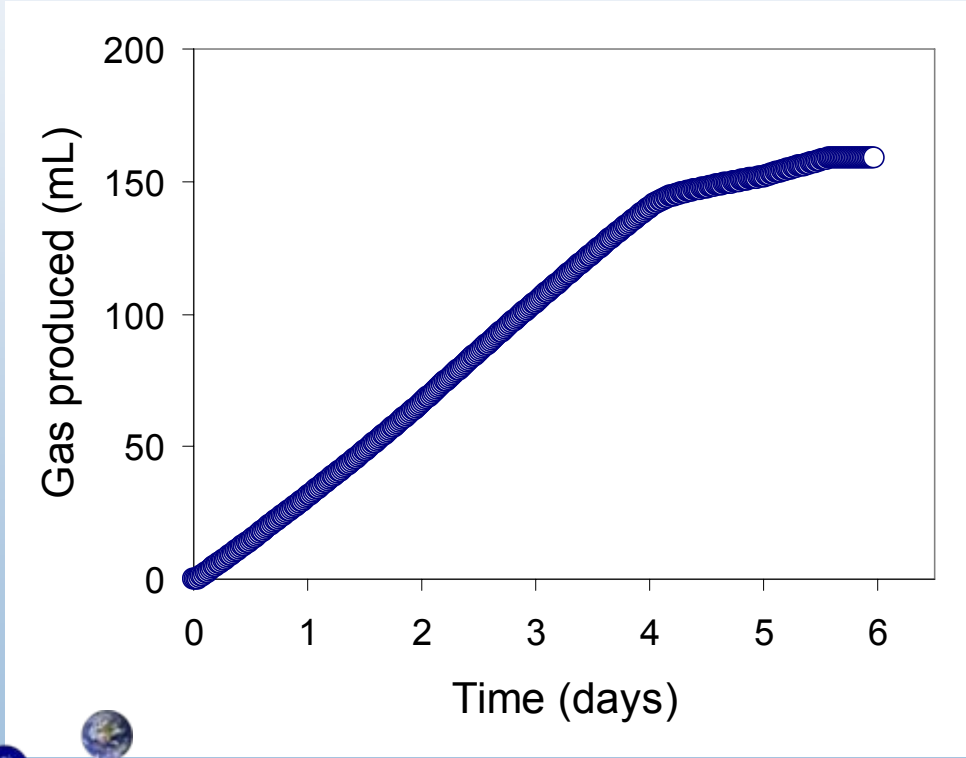
Task 3: Successfully produced H_2 gas from synthetic solution of fermentation end products



- Gas production
  Total= 159 mL
  H_2= 106 mL
- Conversion efficiency= 30 mL H_2/gCOD*
- COD removal=93% (3.6 g-COD)
- Time for cycle: 6 to 7 days
- Problems:
  - Methane gas production
  - Increased CH_4, decreased H_2

*COD: chemical O_2 demand

Meeting Milestone (4/08)

Technical Accomplishments:
Adapted Culture in MEC

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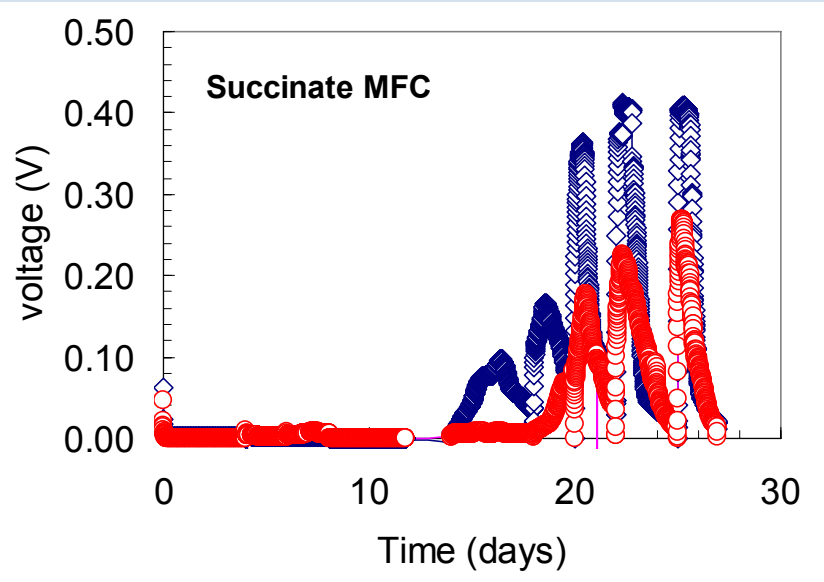
- **Task 3:** Developed acclimated cultures to individual compounds to improve yield and efficiency

To increase H_2 yields, reduce methane production, reactors are being acclimated to individual compounds.

(Duplicate reactors shown below)



- Reactors first run in microbial fuel cell mode (MFC); and switch to MEC mode later.

- Successful acclimation, with maximum voltage of:

  - Acetate: 556 mV
  - Lactate: 543 mV
  - Ethanol: 523 mV
  - Succinate: 412 mV
  - Formate: 228mV

Technical Accomplishments:
Testing Xylose Feedstock

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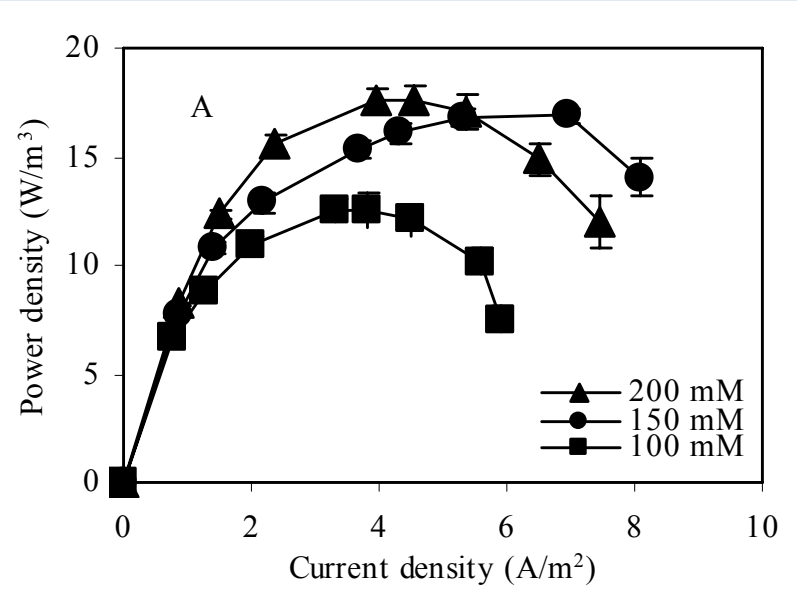
Task 3: Examined electricity production using **xylose** (major sugar of hemicellulose) at different concentrations and solution ionic strength



- Work primarily supported by visiting researcher at Penn State
- Provided an opportunity to examine implications of bioenergy production using an alternative feedstock, and effects of scale-up
- Produced 13 W/m^3 (673 mW/m^2) at Coulombic efficiencies of 61-85% in a medium-scale reactor (0.8 L) at 100 mM ionic strength, with slightly higher power in other solutions.

These results will be useful in considering scale up of MEC systems using hemicellulose

Future Work: Task 1 (NREL)

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- Optimize bioreactor performance for scale-up fermentation of corn stover
- Determine H_2 molar yield, carbon mass balance, and profiles of metabolites (milestone 8/08)
- Provide above fermentation waste products for Penn State MEC process for additional H_2 production
- Test other pretreated feedstock, i. e., switch grass
- Develop continuous (vs batch) fermentation with cellulosic substrates

Future Work: Task 2 (NREL)

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- Test other metabolic pathway inhibitors in improving H_2 yield (Milestone 6/08)
- Combine inhibitors for cumulative improvement
- Optimize growth of *C. thermocellum* on agar plates
- Develop genetic methods for pathway engineering
  - collaborate with Univ. Manitoba to accelerate progress and leverage DOE funding)
- Test scale-up fermentation using metabolic pathway inhibitors for improving H_2 molar yield

Future Work: Task 3 (Penn State)

- Acclimate cultures to all components in the synthetic fermentation product in MEC to improve yield and efficiency
- Test adapted culture using all components
- Use actual biomass fermentation waste products provided by NREL

Summary

DOE Hydrogen Program

- Growth conditions have been optimized for *Clostridium thermocellum* using various cellulosic substrates (cellulose, corn stover)
- Identified key metabolic pathways to block to improve H_2 yield
  - Improved H_2 yield up to 81%
  - Provide a knowledge-based approach to guide metabolic pathway engineering
- H_2 has been successfully produced in MEC using synthetic fermentation waste products